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THE STATE OF EX-SITU CONSERVATION IN NIGERIA

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Abstract

Ex-situ conservation is the process of protecting an endangered species of plant or animal by removing part of the population from a threatened habitat and placing it in a new location, which may be a wild area or within the care of humans. There are several methods of ex-situ conservation being practiced in various parts of the world. However, the ex-situ conservation methods used in Nigeria include botanic and zoological gardens, arboretum, genebanks and in-vitro storage, a few DNA banks and cryopreservation efforts, and there are no active pollen banks. This paper reviews the state of the ex-situ conservation sites in Nigeria in order to bridge the information gaps on the ex-situ conservation of genetic resources in Nigeria. The research institutions, Universities and relevant non-Governmental organizations involved in the conservation of genetic resources ex-situ were taken into account, while their germplasm collections were stated. The challenges faced by ex-situ germplasm conservation were discussed and the role of the Government in improving those situations was emphasized.

Keywords: *Ex-situ; botanic gardens; genebanks; DNA bank; cryopreservation; arboretum; pollen bank; in-vitro bank.*

Introduction

Ex situ conservation deals with protection of biological diversity components outside their natural habitats [1]. It is the process of protecting an endangered species of plant or animal by removing part of the population from a threatened habitat and placing it in a new location, which may be a wild area or within the care of humans [2]. The maintenance of viable and self-sustainable populations of wild species in their natural state represents the ultimate goal, but habitat destruction is inevitable and endangered species need to be preserved before they become extinct. In general, *ex situ* conservation, which literally means, "off-site conservation is applied as an additional measure to supplement *in situ* conservation. Biological diversity conservation *ex situ* is therefore complementary to *in situ* conservation and can act as an "insurance policy" when species are threatened in their natural habitats. The purpose of ex situ conservation stands is to keep genetic resources in a secure area for future utilization [3]. *Ex situ* conservation provides the opportunity to study the biology of and to understand the threats to endangered species, in order to eventually consider successful species recovery programs, which would involve restoration and reintroduction. It also has the advantage of preserving plant material and making it available for research purposes, without damaging the natural populations. Moreover, ***o****ther* purposes of ex-situ conservation include rescuing threatened germplasm, to produce material for conservation biology researches, to bulk up germplasm for

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storage in various forms of ex situ facilities, to supply material for various purposes to remove or reduce pressure from wild collections, growing species with recalcitrant seeds that cannot be maintained in a seed store, to make available material for conservation education and display and to produce material for reintroduction, reinforcement, habitat restoration and management. *Ex situ* conservation methods sample the genetic diversity of species by using certain criteria and they store/propagate the collected material outside the natural environments in which the species grow [4]. The importance of *ex situ* collections for conservation in situ was realized when collections in botanical gardens and arboreta helped in the implementation of population management and recreation [5-7].

Ex situ germplasm conservation is done in live gene banks (botanical gardens, arboreta, home gardens, horticultural centres and field gene banks in research stations), as stored seeds in conventional seed banks, or as *in vitro* storage of plant tissue (buds, meristems, calluses etc). In Nigeria, apart from educational institutions (universities etc) and research institutions and centers (with specific crop mandates), active *ex situ* gene banks in the form of botanical gardens hardly exist apart from those in city centers, commercial horticultural outfits, dot road sides in some big urban centers, selling largely introduced ornamental plants [8]. Africa holds at least 25% of the global pool of Plant Genetic Resources [9].

This paper aimed to compile the state of ex-situ conservation sites in Nigeria, their collections and to identify the areas that require improvement in the management of germplasms in Nigeria.

Botanic Gardens

A botanic garden is an institution holding documented collections of living plants for the purpose of scientific research, conservation, display and education [10]. The history of botanic gardens can be traced back to as far as the Hanging Gardens of Babylon, built by King Nebuchadnezzar in 570 BC as a gift to his wife. Early botanic gardens were designed mainly for the purpose of recreation. By the 16th century, they had become important centers for research, as they promoted the study of taxonomy and became focal point for the study of aromatic and medicinal plants and, more recently, they have taken on significant conservation responsibilities. Apart from their living collections, botanical gardens often have herbaria and carpological collections and an increasing number have seed banks and *in vitro* collections.

There are over 2500 botanic gardens in 150 countries across the world holding over 6 million accessions of living plants representing around 80,000 species [11], as well as 250,000 seed bank accessions [10]. In general, botanical gardens focus on conserving the inter-specific diversity of flora and thus, tend to maintain a large number of species with relatively few accessions for each species. In its country report, China indicated that it had 170 botanical gardens and India reported 150. The Russian Federation reported that it had about 75 botanical gardens, Germany 95, Italy 102, Mexico 30 and Indonesia 12 [12]. There are about 85 botanic gardens in Africa. They are involved in identification and classification (taxonomy) of plant species, propagation of plants and frequent gene sampling [9].

Among the Western, Central and Eastern African countries, Nigeria has the highest number of botanic gardens, 16, followed by Kenya with 9, Ghana and Senegal, with 5 each, Cameroon with 4 among others, which adds up to 61 botanic gardens in the region [13]. However, recent surveys for Botanic Garden Conservation International (BGCI) by the author revealed that there are about 20 botanic gardens in the country. In Nigeria, botanic gardens are owned by Departments of Botany or other related Departments in Universities, state Governments, NGOs and other relevant institutions. Table 1 gives a list of selected botanic gardens in Nigeria.

The University Botanic Garden in University of Ibadan is the oldest botanic garden, being established in 1948. Furthermore, there is the need to establish a central coordinating institution for all the botanic gardens in Nigeria; currently, there is no central coordination for all those botanic gardens.

Table 1. Main Botanic Gardens in Nigeria

S/N	Botanic Gardens	Location	Ownership
1	University Botanic Gardens	Ibadan	University of Ibadan
2	University Botanic Gardens	Nsukka	University of Nigeria, Nsukka
3	University Botanic Gardens	Lagos	University of Lagos
4	Agodi Gardens	Ibadan	Oyo State Government
5	Abuja Gardens	Abuja	Federal Capital Territory
6	Kano Botanic Gardens	Kano	Bayero University, Kano
7	Murtala Mohammed Botanic Garden	Lagos	General Murtala Mohammed family
8	Calabar Botanic Garden and Conservation Centre	Calabar	Iroko Foundation/ Cercopan/ Cross River State Government
9	Shodex Botanic Garden	Lagos	Shodex Beautification Landmark Ltd
10	Sarius Palmetum and Botanic Garden	Abuja	Mrs. Murtala Mohammed
11	Ahmadu Bello University Botanic Garden	Zaria	Ahmadu Bello University
12	University Botanic Gardens	Minna	Federal University of Technology, Minna
13	IITA Arboretum	Ibadan	IITA
14	University Botanic Gardens	Port Harcourt	University of Port Harcourt
15	University Botanic Gardens	Calabar	University of Calabar
16	University Botanic Gardens	Sokoto	Usman DanFodio University
17	Makurdi Botanic Garden	Makurdi	Benue State Government
18	University Botanic Gardens	Abeokuta	Federal University of Agriculture, Abeokuta
19	University Botanic Gardens	Bauchi	Abubakar Tafawa Balewa University
20	Ondo State Biological Garden	Akure	Ondo State Government

Zoological Gardens

Today zoological gardens/zoos provide an opportunity to open up a whole new world of curiosity and interest in the natural world and sensitize visitors to the value and need for the conservation of wildlife. Zoos were initially started for the entertainment of people. Gradually over the years, they have come to play an important role in conservation. The ultimate goal of zoos is the conservation of animals that are endangered in the wild. Zoos all over the world receive approximately 600 million visitors annually and their potential for making people of all ages aware of the threats to the global ecology is unlimited [14]. The enormous number of people reached by the zoo network, coupled with the global perspective in which each individual zoo presents the elements of nature, results in great potential for global conservation awareness.

There are 6 recognised zoological gardens in Nigeria, which is the second largest number in Africa, after South Africa with 12 zoological gardens (www.zoos-worldwide.de). Nigerian zoological gardens include Abuja Children's zoo (Abuja), Audu Bako zoo (Kano), Enugu zoo (Enugu), Ibadan University zoo (Ibadan), Jos Wildlife Park (Jos) and Sanda Kyarimi zoo (Maiduguri). However, there are many other zoological gardens in Nigeria with a significant number of conserved animals. Universities such as University of Lagos, Obafemi Awolowo University and many other Universities with Departments of Zoology, have over the years, developed their zoological gardens as well. Furthermore, many research institutions have animal sections for rearing, breeding and conserving animals such as cane rats, fish, sheep, goats, domestic fowls, snails, rabbits, birds and many other animals. Such Nigerian research institutions include the National Centre for Genetic Resources and Biotechnology (NACGRAB), National Animal Production Research Institute (NAPRI), Institute of Agricultural Research and Training (IAR&T), National Veterinary Research Institute (NVRI); moreover, there are many other tertiary institutions – universities and colleges of forestry, wildlife and/or animal production – with departments of animal science, and/or departments of wildlife and other related fields, which also have relatively small animal units for the study and conservation of animals. Many of the zoos across the country are not well catered for, with animals that are dying of starvation.

Related to Zoological gardens are aquaria. However, only 4 African countries have public aquaria – Egypt, Namibia, Morocco and South Africa. Nigeria does not have a globally recognised aquarium. Nevertheless, there are research institutions with mandates on aquatic studies – National Institute for Freshwater Fisheries Research, New Bussa, National Institute for Oceanography and Marine Research, Lagos and also a related Federal College. It is possible for those institutions to have fish ponds and lakes, but more works need to be done, particularly for the establishment of standard aquaria.

Arboreta

An arboretum is an area containing living collections of trees only for conservation and scientific study. An arboretum is different from a botanic garden because the latter may contain all plant forms, while an arboretum deals only with trees. There are no globally recognized arboreta in Nigeria. Nevertheless, some research institutions have designated areas called arboreta, including Forestry Research Institute of Nigeria (FRIN), in addition to several Departments and Colleges of Forestry across the country with several arboreta for teaching and research purposes. Again, just like botanic and zoological gardens, there is no central coordination for all these arboreta, especially one recording their collections, helping to eliminate work duplication and enhancing collaborations among them.

Genebanks

Over the years, genebanks have been established in a number of countries and the number of accessions conserved in about 1400 genebanks now exceeds six million [15], but it is now known to be more than 1,750 individual genebanks [12]. Genebanks conserve genetic resources. But genebanks are not built just to conserve genetic resources; they are intended to ensure that these resources are used, whether it is in farms, breeding programs or in research institutions. Genebanks are located on all continents, but there are relatively few in Africa compared to the rest of the world. Among the largest collections are the ones established over more than 35 years by the CGIAR, which held in trust for the world community [16]. There are less than 35 national gene banks in Africa [9]. Genebanks can either be seed genebanks for conserving plants with orthodox seed behavior or field genebanks for plants with recalcitrant seed behavior.

Seed genebank

The most widely used and most convenient technique for conserving plant genetic resources is seed banking [17]. Seeds are dried to lower moisture contents and are stored at sub-zero temperatures in cold stores or deep freezers. Thus, they can be stored for several years. Therefore, following collection, reliable seed banks must be put in place for conservation of the collected samples. It must be emphasized that regular checks are carried out to test the viability of the stored seeds periodically. The seed bank will serve as a major insurance against permanent loss of any species that had been previously collected [18]. According to FAO, this technique accounts for 90% of the 6 million accessions conserved ex-situ globally [19-20]. The main advantage of seed banking is that it allows large populations to be preserved and genetic erosion to be minimized by providing optimum conditions and reducing the need for regeneration [6].

Seed genebanks are generally much more widespread than field genebanks in the continent. Ethiopia, for example, reported having 60,000 accessions in its national seed genebank and 9,000 in its field genebank. Burkina Faso, the Niger and Zambia all reported having many more accessions in their seed genebanks than in their field genebanks [16].

Seed gene banks in Nigeria are used for the conservation of orthodox seeds, mainly vegetables and cereals, which are used as food crops. All major food crops in Nigeria have been given to the research institutions as national mandate crops for their selection, breeding and improvement. Those research institutions are expected to have genebanks for seed storage.

Additionally, NACGRAB has a modern seed genebank for the conservation of all the orthodox seeds. Through Government policies, all the research institutions are expected to send duplicate samples of their collections to NACGRAB for conservation. Table 2 lists the national research institutions working on plants with orthodox seeds.

Table 2. National Research Institutions and their collections

S/N	Name of Research Institute	Mandate crops	Collections (accessions)
1	National Cereals Research Institute	Rice, sugarcane, soybean, beniseed, acha, castor	Sugarcane – 467 *Oryza sativa* – 200 Oryza glaberrima – 95 Sesame – 82 *Digitaria exilis* – 7 *Digitaria iburua* – 6
2	Institute of Agricultural Research	Sorghum, maize, cowpea, groundnut, cotton, sunflower	Unknown
3	Institute of Agricultural Research and Training	Kenaf, jute, cowpea, maize	Unknown
4	Lake Chad Research Institute	Wheat, millet, barley	*Pennisetum glaucum* – 1000 *Pennisetum glaucum* ssp *monodii* – 40 Triticum spp. – 1000

Collections at NACGRAB seed genebank come from exploration activities, donations and safety duplicates from other research institutions. As of 2010, the NACGRAB seed genebank had 1,757 accessions of cereals, 585 accessions of legumes and 549 accessions of vegetables. In addition, the International Institute of Tropical Agriculture (IITA) genebank has 15,122 cowpea accessions, 1,827 Bambara groundnut accessions, 1,615 *Vigna* wild relatives accessions, 1,754 soybean accessions, 150 African yam bean, 600 accessions of other legumes and 876 maize accessions. Lots of advancement is still needed in seed banking and management in the Nigerian research institutes. That includes the use of the Probit analysis to predict seed longevity and the use of a Seed Digital Imaging computer software to predict the morphological characters of a seed germplasm. The use of those advanced technologies in Nigeria seed genebanks ranged from no use to very little use thereof. Furthermore, routine regeneration, characterization and seed viability testing should be done for all of the conserved germplasms. In many cases, less than 25% of the accessions stored at the genebanks were tested for viability [21].

In addition to seed genebanks, there are also Strategic Grains Reserve Complexes and Community Seed Banks in Nigeria. Figures 1 and 2 illustrate the Strategic Grains Reserve Complexes and their locations in Nigeria. The existing Strategic Grains Reserve Complex in Nigeria has a storage capacity of 325,000 tons of grains.

Fig. 1. Silos of the National Strategic Grain Reserve Complex

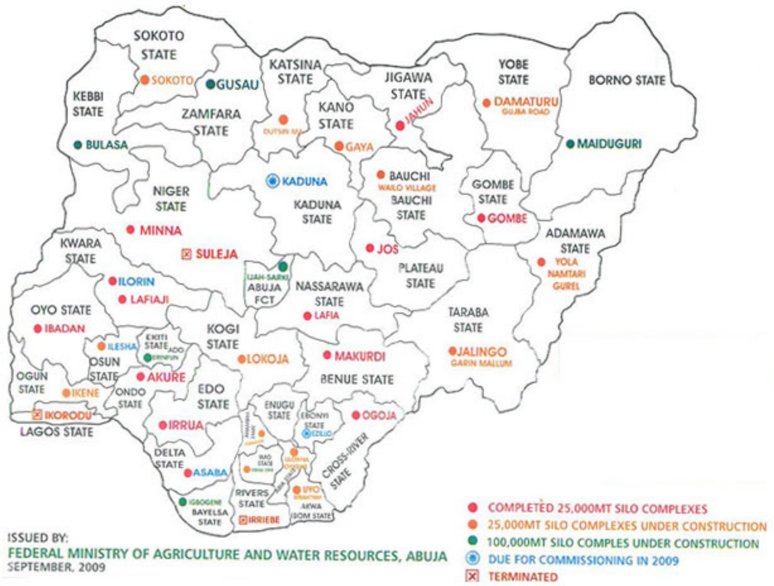

Fig. 2. Locations of the National Strategic Grain Reserve Complexes in Nigeria

Furthermore, the National Agricultural Seed Council (NASC) also conserves and multiplies foundation seeds of newly released crop varieties for onward distribution to farmers. NASC currently has hundreds of crop varieties in their collections.

Organized farmers associations, especially in farm settlements, may have their own Community seed banks, in which excess harvests and farm produce are preserved in a low tech system to be used as planting material in coming planting seasons. The majority of such community seed banks are managed by individual farmers by using indigenous conservation methods. Such indigenous methods include smearing seeds with kerosene, storing them in bottles and adding dry pepper fruit/powder, sun-drying. Maize cobs are tied together and hung above the cooking fire in the local kitchen so that the heat can expel storage pests many other local conservation methods. Those community seed banks and the institutions that support them are extremely important in the preservation of local varieties and for agricultural production.

Field genebank, also called Crop Type Collection Center (CTC)

The conservation of germplasm in field genebanks involves the collecting of material and planting it in the orchard, or field, in another location. Field genebanks have been used for perennial plants including species that produces recalcitrant seeds, species that produces little or no seeds, species that are preferably stored as cloning material and species that have a long life cycle to generate breeding and/or planting material. At the field genebank, the collected plant species are planted out in an orderly manner – according to species, variants and family. The field genebank should be well protected against fire as that can lead to a total loss of the conserved species. The management and maintenance of the field genebank should also be treated with high priority, because it is capital intensive [18].

There are only 1 field genebank in Nigeria, owned and managed by NACGRAB. However, in addition to that, some of the research institutions have orchards which are demonstration plots for their mandate perennial crops. Therefore, such banks could be technically classified as field genebanks. Table 3 shows the list of such national research institutes and their collections. In addition, almost all States of the Federation have commercial orchards under their Ministries of Agriculture where they grow and sell ornamental, woody plants and perennial food crops – such as plantain, pineapple, citrus – to the general public. Furthermore, some private organizations and agro-based companies have plantation orchards of tropical fruits and other perennial food crops for commercialization, which could also be grouped under field genebanks.

Table 3. Research institutes and NGOs with field genebanks and orchards

S/N	Name of Research Institute	Mandate crops	Collections (accessions)
1	National Horticultural Research Institute	Tropical fruits (citrus), banana/plantain, mango, pineapple, indigenous fruits (walnut, Giant star apple, African bush pear, locust tree, hog plum, avocado pear, bush mango), vegetables (tomato, grain amaranth, leaf amaranth, *Corchorus olitorius*, Egusi Melon, okra, pepper, *Telfaria occidentalis*, *Gnetum* spp), spices and ornamental plants.	Citrus – 210 Mango – 17 Pineapple – 2
2	National Root Crop Research Institute	Cassava, yam, potato, sweet potato, cocoyam, ginger	
3	Cocoa Research Institute of Nigeria	Cocoa, Kola, Cashew, Tea, coffee	Cashew – 1,025 Tea – 33 *Coffee arabica* – 100 *Coffee canephora* – 65
4	International Institute of Tropical Agriculture	Cassava, yam, cowpea, maize, bambara groundnut, soybean, African yam bean and other food crops	Yam – 1,067 Cassava – 1,502 Musa – 275
5	Forestry Research Institute of Nigeria	All forest tree crops	*Parkia biglobosa* – 15
6	Nigerian Institute for Oil Palm Research	Oil palm, coconut, date palm, shea butter, raphia palm and other palm.	*Cocos nucifera* – 16
7	Rubber Research Institute of Nigeria	Para rubber, gum Arabic and other latex-producing plants	
8	National Centre for Genetic Resources and Biotechnology	All recalcitrant plants	
9	Centre for Environment, Renewable Natural Resources Management, Research and Development	Forest tree crops and other indigenous plants	*Irvingia gabonensis*- 18 *Dacryodes edulis*- 10 *Vernonia amygdalina*- 40

NIHORT has a broad germplasm collection of citrus fruit species and accessions drawn from different parts of the world, ranging from sweet orange, mandarins, tangelo, grape fruit, lemon, lime, tangor, sour orange and *Poncirus trifoliata*. Other fruits germplasm of plantain (*Musa* spp. AAB - 16 accessions), Banana (*Musa* spp. AAA – 6 accessions), Mango (*Magnifera indica* - 23 accessions), Pawpaw (*Carica papaya* - 14 accessions), Guava (*Psidum guajava* - 67 accessions), Avocado (*Persea americana* - 8 accessions), *Irvingia* nut (16 accessions) and *Treculia* nut (6 accessions), had been collected from within and outside Nigeria and maintained at the institute's headquarters as well as in substations in various agro-ecological zones of the country [22]. Nevertheless, NIFOR also established and maintained a palmetum where representative palms from various areas of the world were included. Many of those palms were found to have ornamental values. So far, not less than 114 different species and accessions have been introduced [23].

An inventory of the NACGRAB Field genebank was done by Borokini et al. [24] and 361 species were listed. NACGRAB field genebank has about 500 accessions spread across the over 300 plant species. It should be noted that NACGRAB has the largest ex-situ collections of the endangered *Irvingia gabonensis* and *Irvingia wombulu* – 64 and 32 accessions respectively – collected from various parts of West Africa, and each accession replicated 10 times on the field genebank. Furthermore, ex-situ conservation works were started for *Parkia biglobosa* in 1993 and 94 accessions were collected and conserved in the seed gene bank in FRIN, 54 of which were collected in other West African countries [25].

DNA bank

A DNA bank is a repository of DNA used for genetic studies, taxonomy, conservation and other purposes. Molecular marker technologies are based on DNA analysis and total DNA, as crude extracts, will be generated for all target samples [26]. Moreover, samples may also remain as amplification products as a result of applying technologies based on the polymerase chain reaction, e.g. random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) [27]. Other technologies, prior to the identification of the markers, require the construction of libraries, i.e. collections of segments of DNA (genomic, c-DNA) containing several copies of the genome of a given species. It is now routine to keep leftover DNA samples or libraries, even after a particular research project has been finished, because they constitute a valuable reserve for research and are a way to continue capitalizing on the investment (time and funds) already made to develop knowledge and/or products [26]. DNA resources can be maintained at -20ºC for short- and mid-term storage (i.e. up to 2 years) and at -70ºC or in liquid nitrogen for longer periods. Nevertheless, those DNA storage activities generally are not planned, being spinoff activities of various ongoing projects. That situation has prompted the consideration of DNA collections as a genetic resource [28]. The major DNA banks in the world include the Frozen Zoo of the Zoological Society of San Diego with DNA specimens of about 7000 endangered animal species, The Royal Botanic Garden (20,000 plant DNA specimens), The US Missouri Botanic Garden (20,000 plant DNA specimens), The Australian Plant DNA bank, The DNA bank at the Leslei Hill Molecular Systematics Laboratory of the National Botanical Institute, Kirstenbosch, South Africa (443 DNA specimens of South African flora) and the DNA bank at the National Institute of Agrobiological Sciences (NIAS), Japan [26]. DNA banks are very few in Africa, with only a few in South Africa. Nigeria is yet to have a functional DNA bank as Nigeria is just developing Molecular Biology facilities in some of the research institutions such as NACGRAB, Sheda Science and Technology Complex (SHETSCO) and National Biotechnology Development Agency (NABDA). In NACGRAB, molecular studies - isolation and purification of genomic DNA, verification of DNA quality by electrophoresis, amplification by Polymerase Chain Reaction (PCR) and gel electrophoresis of PCR products – have started on 150 Sorghum accessions, 7 *Khaya grandifoliola* accessions, one accession each of *Vernonia amygdalina*, *Ixora coccinea* and *Manihot esculenta*, as well as blood samples from domestic fowl, goat, pigs and fish and extra DNA samples of plants and animals constitute the DNA bank maintained at -40ºC. Recently, the Consortium for the Barcode of Life, CBOL, an international initiative promoting the use of DNA bar-coding tool as a global standard for species identification, is introducing this technology into Nigeria with University of Lagos and NACGRAB among the participating institutions.

Pollen bank

Pollen storage is important for germplasm conservation, exchanges and handling and it enhances breeding efficiency [29]. The storage of pollen at low temperatures as a means of conserving plant genetic resources has been widely discussed by many authors, for various species [30-33]. Long-term pollen storage is important for germplasm preservation, pollen research, germplasm exchange, and improved efficiency in plant breeding. Significant progress has been made in maintaining the viability of stored pollen from agronomic crops in at least 17 genera [34]. Pollen can be stored in liquid air or freeze-dried and sealed in vacuumed glass capsules, or in nitrogen gas [30]. It is possible to store pollen of many species at temperatures between 4°C and –20°C for short-terms. Long-term viability can be maintained by storing pollen at –80°C or LN temperatures (–196°C) [34]. A more general advantage of pollen grains over seeds is their relatively smaller size. The average diameter of pollen grains is 30-40 um, which means that very large numbers can be collected and conserved [35]. Quite apart from the obvious advantage of better genetic representation, the access to vast numbers of haploid individuals enables the breeder to make use of pollen selection for polygenic traits from the very first pollination. Pollen preservation may be useful for base collections of species that do not produce orthodox seeds. It requires little space but some cytoplasmic genes would be lost. Like seeds, pollen can be divided into desiccation tolerant and intolerant. However, information about storage characteristics of pollen from wild species is fragmentary, existing mainly for some crop relatives and for medicinal and forest species [36]. Pollen conservation is advantageous since pollen can be stored for a long period without losing its viability [34]. In this way, transportation and diffusion of pollen in view of carrying out hybridization between plants dispersed in space and time at flowering will be facilitated [37]. But pollen germination and viability depend on many factors involving culture medium composition [38-39] and air pollution conditions [40].

In spite of the breeding work going on in several research institutions, there is no pollen bank in Nigeria. Often, excess pollen grains from breeding work are discarded, while palynologists prepare temporary and permanent microscopic slides of pollen grains for analysis, but most of the pollen grains used are not stored at all. Therefore, there is a need for the research institutions and NACGRAB to establish pollen banks for their mandate crops, for breeding studies and conservation and also to conduct extensive studies on pollen behavior for different plant species in Nigeria, for effective pollen conservation.

In-vitro banks

Germplasm conservation of vegetatively propagated crops (e.g. banana, plantain, yam, cassava etc) forest species, especially those with recalcitrant (hard to store) seeds (e.g. mango, cocoa, *Symphonia globulifera*, *Irvingia gabonensis*) in field genebanks poses tremendous problems in terms of required land areas and labour input during annual or perennial replanting, testing and documentation. Such collections are also exposed to threats from biotic and abiotic stress agents. Consequently, *in vitro* conservation is recommended, at least as a supplement to field collections, as long as an adequate protocol for micropropagation has been worked out for the species [41]. The advantage of *in vitro*, or reduced growth storage, include little space necessary in growth rooms for maintaining thousands of genotypes and the absence of diseases and pest attack in culture vessels. Furthermore, *in vitro* storage eliminates the need for long and frustrating quarantine procedures during the movement and exchange of germplasm. Virtually any part of the plant could be used as explants in establishing cultures for storage, although the best results have been obtained by using apical meristems, axillary buds, embryos and gametes. Several nutrient media are used in *in vitro* cultures, most common of which being the Murashige and Skoog (MS) nutrient medium. During *in vitro* storage the growth of the culture is slowed down through one, or a combination of several methods, such as reducing the concentrations of the minerals, or by using media with lower salt concentrations [42]; using low incubation temperatures [43-45]; addition of osmotica [42,44,45]; reduction of the gas pressure in culture vessels [46] and varying the light regime [47]. Conservation *in-vitro* depends entirely on the techniques of plant cell, tissue and organ culture and is appropriate in situations where conventional seed storage cannot, or is not to be employed. The material stored *in vitro* may be protoplast, isolated cells grown in suspension or on semi-solid medium, meristem cultures at various stages of development, or organized plantlets.

Tissue culture and in-vitro propagation of plants have been developed in Nigeria to a limited stage. Currently, micrografting, protoplast fusion, protoplast isolation, fusion and culture, biological nitrogen fixation and culturing of organs, nucellus and endosperm are yet to be demonstrated in any Biotechnology laboratory in Nigeria, as only relatively few indigenous plants in Nigeria have tissue culture protocols developed for them. Since the tissue culture "revolution" in Nigeria in early 2000, almost all the Research institutions in Nigeria are scrambling to establish tissue culture laboratories. Some of them with in-vitro laboratories include NACGRAB, NCRI, NIHORT, FRIN, NRCRI, NIFOR and NABDA among others, with a more standardized tissue culture laboratory in IITA. NACGRAB has developed tissue culture protocols for several crops which include pineapple, plantain, cassava, *Jatropha curcas*, *Solanum* sp., sweet potato, *Vernonia amygdalina*, Eucalyptus, sugar cane, *Azadirachta indica* and date palm among many others. However, majority of these plantlets were produced for mass propagation and not for conservation. Again, the major challenge faced in these laboratories is contamination of nutrient media and plant materials. In addition, a relatively new technology for commercial micropropagation, Temporary Immersion Bioreactors (TIBs), was recently introduced to Nigeria via NACGRAB and SHETSCO, through which thousands of plantlets can be propagated at the same time.

Cryopreservation

Cryopreservation is an attractive alternative for the storage of base collections and involves the freezing of plant and animal cells, usually to the temperature of liquid nitrogen (-196ºC), at which point cell division and consequently growth and all other biological activities are completely arrested. This must be done in such a manner that viability of the stored material is retained and biological functions and growth can be reactivated after thawing [48-49]. There are several cryopreservation techniques available, such as slow cooling, rapid freezing, vitrification and encapsulation-dehydration methods [50]. Liquid nitrogen storage is useful for the preservation of various types of plant material including whole seeds, embryos, suspension cells, callus, protoplast cultures, gametes and meristems. Cryopreservation involves the storage of plant material at ultra-low temperatures in liquid nitrogen (-196°C). At this temperature, cell division and metabolic activities remain suspended and the material can be stored without changes for long periods of time. Cryopreservation is the only available method for long-term conservation of vegetatively propagated plant germplasm. The choice of material includes cells, protoplasts, shoot apices, somatic embryos, seed or excised zygotic embryos. Cryopreservation requires limited space, protects material from contamination, involves very little maintenance and is considered to be a cost-effective option [51].

Ajayi et al. [52] reported the successful storage of *Telfairia occidentalis*, a Nigerian indigenous leaf vegetable by using the cryopreservation technique. Chaudhari and Mshelia [53] reported the storage of cattle and buffalo semen, while the collaborative NACGRAB – Department of Animal Sciences (Obafemi Awolowo University) project on the cryopreservation of African catfish sperm was also reported to be successful [54]. Several other cryopreservation studies have been carried out in various research institutions and Universities across the country, many of which have been published in research paper journals, but most of those studies were limited to few months of cryopreservation, after which the experiments were terminated. The national technical capacity for cryopreservation is significantly limited and largely unsustainable at the moment.

Medicinal plant gardens

The original purpose of the earliest botanic gardens established in Europe in the 16th century was the cultivation and study of medicinal plants, at a time when medicine and botany were essentially the same discipline [11]. A medicinal plant garden is a botanic garden designated for the cultivation and conservation of medicinal plants, whose medicinal values are widely known and used. In other words, a medicinal garden can be considered a botanic garden, with focus on medicinal plants only. There have been scientific debates on cultivation of medicinal plants, with some studies supporting the cultivation of medicinal plants as a means of reducing the pressure on wild stocks and for conservation [55-57]. Others pointed out the disadvantages of medicinal plant cultivation [58-61]. Although many botanic gardens in the world have a special section for medicinal plants, some medicinal gardens were also purposely established. In Africa, recognized medicinal gardens exist in Ghana and South Africa.

In Nigeria, the concept of medicinal garden did not get recognition in research institutions until mid 2000. Almost at the same time, NACGRAB and FRIN established medicinal gardens, each containing about 60 medicinal plant species at the moment. However, The Department of Pharmacognosy in Obafemi Awolowo University, Ile-Ife had already established a medicinal flora garden in the 1990s. Furthermore, Nigeria Natural Medicine Development Agency (NNMDA) had also established a medicinal garden which contained approximately 50 medicinal plants within their premises in Lagos, while there are on-going plans to establish medicinal gardens in all the 6 geo-political zones of the country. National Institute for Pharmaceutical Research and Development (NIRPD), Abuja also has a medicinal garden for research purposes. Furthermore, the National Council for Arts and Culture (NCAC), Nigeria also established medicinal (herbal) gardens in 4 states – Kano, Edo, Taraba and Ebonyi – of the Federation and is planning to establish more. According to the NCAC, the herbal gardens are designed to conserve endangered herbal plant species, shrubs and trees of medicinal, economic and social values. Similarly, UNDP is funding the establishment of a medicinal garden in Ipetu-Ijesa, Osun State for the preservation of endangered and rare medicinal plant species. Another medicinal garden in Idena Itoikin, Lagos state recently received the attention of the State Government for rehabilitation, after many years of having gone moribund. There are probably many other medicinal gardens spread across Nigeria which are unknown as of now. NNMDA should be empowered for the central coordination and documentation of all the medicinal gardens in Nigeria.

Problems of maintaining ex-situ conservation sites

The maintenance and management of ex-situ protected areas is the hallmark of conservation efforts. Many African countries, including Nigeria, have reported many challenges in the maintenance of such germplasms.

Electricity is perhaps, the major challenge facing ex-situ collections maintained at low temperatures. Most African countries still have problems with stable electricity, in spite of the huge natural resources at their disposal for generating electricity. This has caused losses of conserved germplasm, especially the seed genebanks. Guinea reported the loss of its entire *ex situ* collection as a result of a failure in the electricity supply [16]. Likewise in NACGRAB Nigeria, more than 50% of its germplasm base was lost due to electricity failures in the past 6 years. Electricity is also a strong determining factor for any African country that wishes to establish DNA bank or cryopreservation tanks.

Moreover, poor management of in-situ protected areas are also evident in some of the ex-situ field collections. For example, Borokini [62] identified 25 invasive alien species in NACGRAB Field genebank alone. The personnel of many in-situ and other similar ex-situ protected areas are not trained in the management of invasive species.

Furthermore, almost all of the Nigerian research institutions and Universities list inadequate funding as one of their challenges. This indeed, is very true and it hampered the regular maintenance of the collections, as for example, the regular regeneration and characterization of orthodox seeds. Worse still, the majority of those institutions could not go on the routine exploration and collection of germplasms across the country to boost their ex-situ collections, because of a shortage of funds.

Many of the high-tech storage facilities are lacking in many of the conservation research institutions. For example, when NACGRAB took off in 1987, many of the seed genebank facilities used were donated by FAO, UNDP and other international organizations, many of which need to be replaced with improved equipment.

Many of the people working with ex-situ conservation efforts in Nigeria are not experts. Hardly will you see a Seed Scientist in charge of Seed genebanks, few or no Universities offer quality education in Biotechnology, Tissue Culture, Plant Genetics, Plant Biochemistry and other relevant courses. Theoretical learning is very common in Universities with little or no hands-on learning experiences, obviously because many of the lecturers too did not have practical experiences and/or there are no facilities for practical classes. Worse still, technical staff recruitment is still characterized by nepotism and favoritism, which got non-qualified people into sensitive offices that manage germplasm. All the above-mentioned would definitely lead to a poor management of genetic resources in Nigeria. Again, most of the genebanks, gardens and institutions that manage them do not have proper documentation of their collections and their movements. Other challenges faced include shortage of staff, pests and diseases.

The Federal Government needs to set up an institutional and policy framework that will assist in monitoring and coordinating the conservation of germplasms. Just as there is a National Park Service that coordinates the activities of all the National Parks, there needs to be a central coordinating unit for all the botanic gardens, medicinal gardens, in-vitro banks, cryopreservation tanks, seed genebanks and all other ex-situ protected sites. While considering this, a central coordinating unit should also be created for all herbaria in Nigeria, as well. All the research institutions conserving genetic resources are just working on their own with very little collaboration among themselves. Furthermore, Nigeria is yet to ratify and sign the Material Transfer Agreement (MTA) so as to allow for access to and exchange of germplasms between Nigeria and other countries.

The urgency for the collection of plant genetic resources in order to fill gaps in collections and to conserve unique diversity before it is extirpated *in situ* has been recognized for decades [63-66] and continues to be emphasized [67-75]. FAO [16] highlights the need for collection, especially for neglected and underutilized crop diversity and for crop wild relatives. Therefore, the relevant institutions must be empowered to embark on regular and collaborative exploration activities, in order to increase their collection bases.

Conclusions

When comparing the high biodiversity in Nigeria and the number of species and accession conserved ex-situ, it is obvious that very little has been done in ex-situ conservation in Nigeria, in spite of the many research institutions, Universities and NGOs that focused on the conservation of genetic resources in Nigeria. Furthermore, proper documentation of collections and management of those protected areas are lacking, while the dwindling electricity supply also makes high tech conservation efforts difficult and challenging. Many of these institutions require improved facilities and technical experts. The Federal Government a lot of roles to play in the management of these conserved germplasms, with adequate funding for specific purposes and by providing central coordination for their activities. All the relevant institutions also need to intensify exploration and collection activities, in order to increase their germplasm base.

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